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Short Communication

Detection of paternal uniparental disomy 9 in a neonate with prenatally detected mosaicism for a small supernumerary marker chromosome 9 and a supernumerary ring chromosome 9

Chih-Ping Chen [a, b, c, d, e, f, *], Ming Chen [g, h, i], Liang-Kai Wang [a], Schu-Rern Chern [b], Peih-Shan Wu [j], Shin-Wen Chen [a], Shih-Ting Lai [a], Shun-Ping Chang [g, h], Chien-Wen Yang [b], Chen-Wen Pan [a], Wayseen Wang [b, k]

[a] Department of Obstetrics and Gynecology, Mackay Memorial Hospital, Taipei, Taiwan
[b] Department of Medical Research, MacKay Memorial Hospital, Taipei, Taiwan
[c] Department of Biotechnology, Asia University, Taichung, Taiwan
[d] School of Chinese Medicine, College of Chinese Medicine, China Medical University, Taichung, Taiwan
[e] Institute of Clinical and Community Health Nursing, National Yang-Ming University, Taipei, Taiwan
[f] Department of Obstetrics and Gynecology, School of Medicine, National Yang-Ming University, Taipei, Taiwan
[g] Department of Medical Research, Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan
[h] Department of Genomic Medicine, Center for Medical Genetics, Changhua Christian Hospital, Changhua, Taiwan
[i] Department of Obstetrics and Gynecology, Changhua Christian Hospital, Changhua, Taiwan
[j] Gene Biodesign Co. Ltd, Taipei, Taiwan
[k] Department of Bioengineering, Tatung University, Taipei, Taiwan

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ABSTRACT

Objective: We present the association of paternal uniparental disomy (UPD) 9 with mosaicism for a small supernumerary marker chromosome 9 [sSMC(9)] and a supernumerary ring chromosome 9 [r(9)].
Materials and methods: A 38-year-old woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Amniocentesis revealed a karyotype of 47,XY,+mar [25]/48,XY,+mar,+r(9) [4]/47,XY,+r(9) [1]/46,XY [6]. The parental karyotypes were normal. Array comparative genomic hybridization (aCGH) of cultured amniocytes revealed a result of *de novo* 9p13.1q21.11 (38,792,472–71,026,063) × 2.64. The marker chromosome was determined to be an sSMC(9) by spectral karyotyping and aCGH. A phenotypically normal baby was delivered at 38 weeks of gestation. During pediatric follow-ups at age two years, the neonate manifested normal psychomotor and growth development. Cytogenetic analysis, metaphase fluorescence *in situ* hybridization (FISH), single nucleotide polymorphism (SNP) aCGH and polymorphic DNA marker analysis were performed on the peripheral blood of the neonate.
Results: The neonate's blood had the following results. Metaphase FISH confirmed coexistence of the sSMC(9) and the supernumerary r(9). The karyotype was 47,XY,+sSMC(9) [14]/48,XY, +sSMC(9),+r(9) [10]/47,XY,+r(9) [6]/46,XY [10]. SNP aCGH revealed arr 9p22.3q21.11 (14,234,165–71,035,608) × 2–3, arr 9p24.3p22.3 (216,123–14,629,321)hmz, arr 9p21.3p13.2 (24,769,722–36,732,597)hmz and arr 9q21.11q34.3 (71,013,799–141,011,581)hmz. Polymorphic DNA marker analysis showed paternal isodisomy 9.
Conclusion: Individuals with mosaicism for sSMC(9) and supernumerary r(9) may be associated with paternal UPD 9.

Introduction

A small supernumerary marker chromosome (sSMC) is an extra structurally abnormal chromosome that cannot be identified by conventional cytogenetics and has a size equal to or smaller than

* Corresponding author. Department of Obstetrics and Gynecology, MacKay Memorial Hospital 92, Section 2, Chung-Shan North Road, Taipei 10449, Taiwan Fax: +886 2 25433642, +886 225232448.
E-mail address: cpc_mmh@yahoo.com (C.-P. Chen).

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that of chromosome 20 [1]. With the advent of molecular cytogenetic technology, prenatal diagnosis of an sSMC(9) has been well described [2–16].

Clinical reports of uniparental disomy (UPD) 9 associated with mosaic sSMC(9) are rare. To our knowledge, only one case with maternal UPD 9 and mosaicism for a supernumerary r(9) (::p12→q10::) in a girl with moderate mental retardation and speech delay but no obvious dysmorphism [5,6,9,14] has been reported. Here, we present a rare case of paternal UPD 9 associated with mosaic sSMC(9) in a neonate with no apparent phenotypic abnormalities.

Materials and methods

Clinical description

A 38-year-old woman underwent amniocentesis at 17 weeks of gestation because of advanced maternal age. Amniocentesis revealed a karyotype of 47,XY,+mar [25]/48,XY,+mar,+r(9) [4]/47,XY,+r(9) [1]/46,XY [6] (Fig. 1). The parental karyotypes were normal. Array comparative genomic hybridization (aCGH) analysis on cultured amniocytes by Roche ISCA Plus Cytogenetic Array (Roche NimbleGen, Madison, WI, USA) revealed a result of *de novo* arr 9p13.1q21.11 (38,792,472–71,026,063) × 2.64 (Fig. 2). The marker chromosome was determined to be an sSMC(9) by spectral karyotyping (Fig. 3) and aCGH. A phenotypically normal baby was delivered at 38 weeks of gestation with a body weight of 3325 g and a body length of 48 cm. During pediatric follow-ups at age two years, the neonate manifested normal psychomotor and growth development. Cytogenetic analysis, metaphase fluorescence *in situ* hybridization (FISH), single nucleotide polymorphism (SNP) aCGH and polymorphic DNA marker analysis by quantitative fluorescent polymerase chain reaction (QF-PCR) assays were performed on the peripheral blood of the neonate.

Conventional cytogenetic analysis

Routine cytogenetic analysis by G-banding techniques at the 550 bands of resolution was performed on the neonate's peripheral blood according to the standard cytogenetic protocol.

FISH

Metaphase FISH analysis on cultured lymphocytes was performed using the bacterial artificial chromosome (BAC) probe of RP11-960P7 (9p22.2, 17,807,208–17,995,652; Dye: Cy5) and Vysis CEP9 Alpha Spectrum Orange (Abbott, Abbott Park, IL, USA) according to the standard FISH protocol.

aCGH

SNP aCGH on the DNA extracted from peripheral blood was performed using CytoScan 750K Array (Affymetrix, Santa Clara, CA, USA). The array has 750,000 probes with a median resolution of 100 kb across the entire genome according to the manufacturer's instruction.

QF-PCR

QF-PCR assay was performed on the DNAs extracted from the peripheral bloods of the neonate and his parents. The markers of D9S304 (9p21.1), D9S303 (9q21.32), D9S249 (9q21.33) and D9S299 (9q31.2) were applied to undertake polymorphic marker analysis to examine the coexistence of UPD 9.

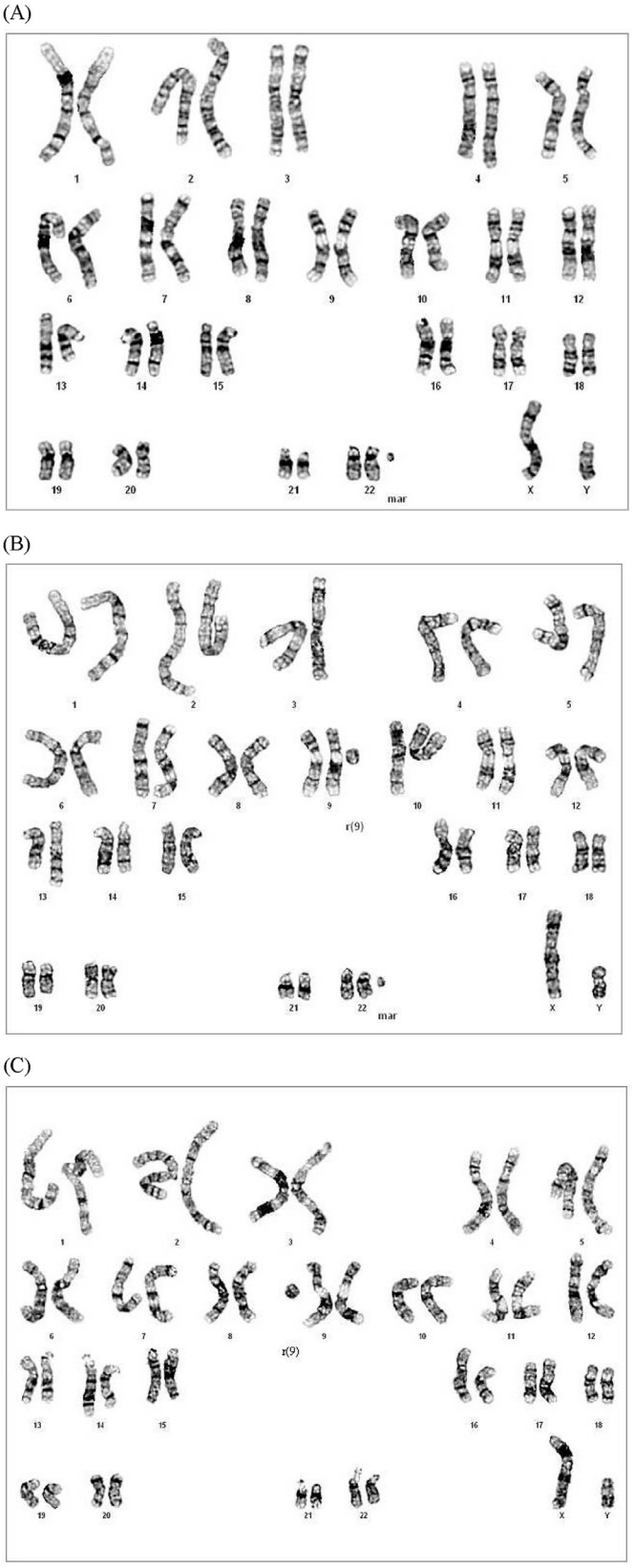

Fig. 1. (A) A karyotype of 47,XY,+mar. mar = marker chromosome. (B) A karyotype of 48,XY,+mar,+r(9). r = ring chromosome. (C) A karyotype of 47,XY,+r(9).

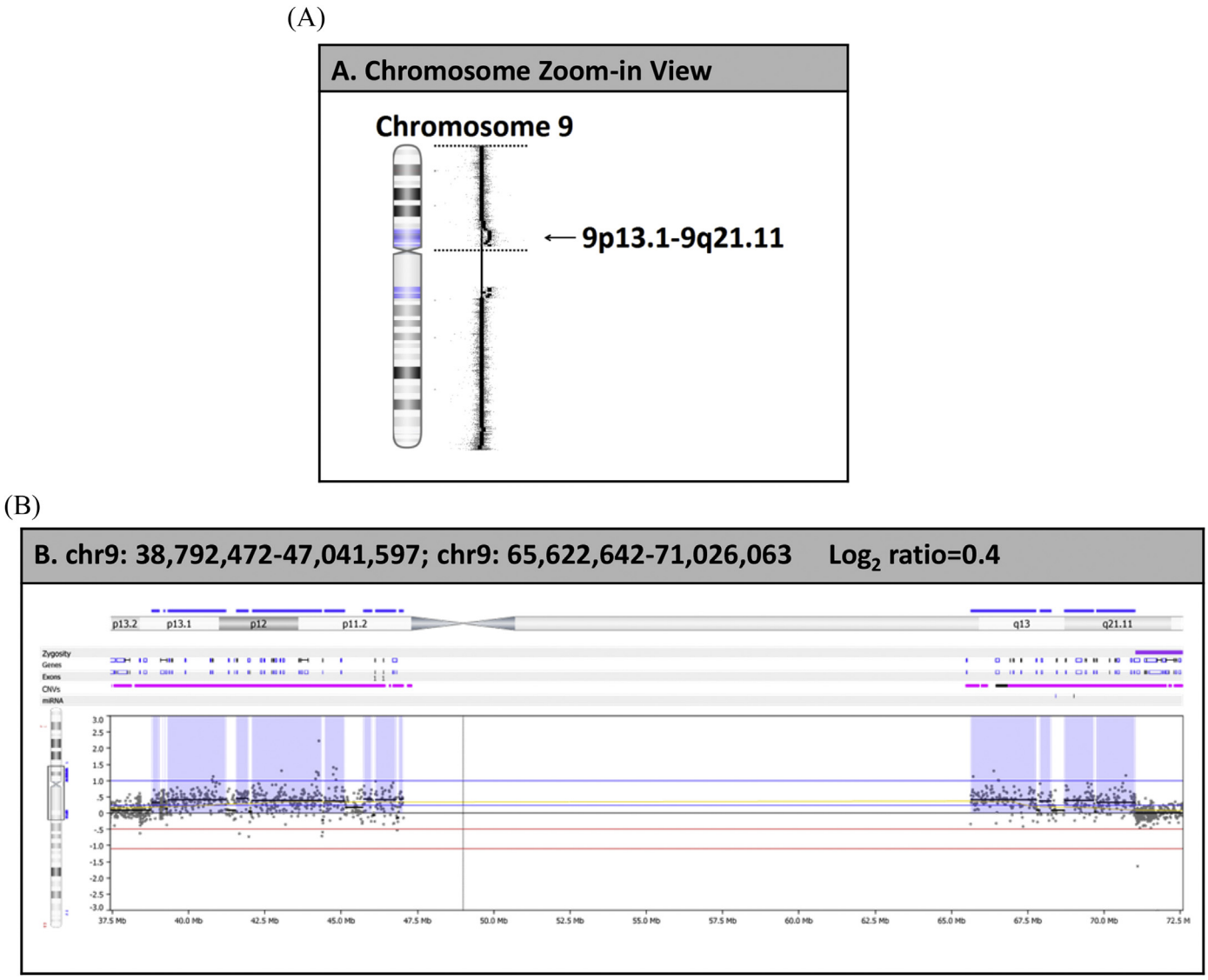

Fig. 2. Array comparative genomic hybridization analysis on cultured amniocytes shows the result of arr 9p13.1q21.11 (38,792,472–71,026,063) × 2.64. (A) and (B) Chromosome 9 zoon-in views.

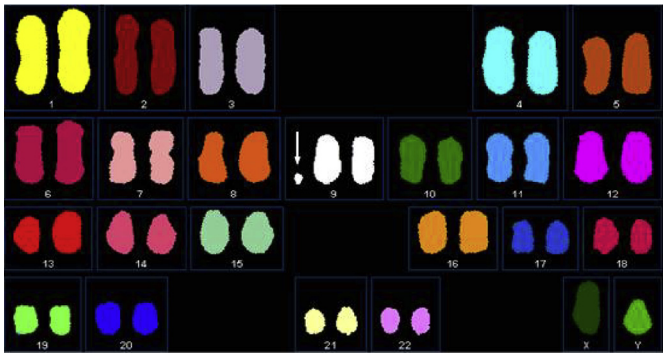

Fig. 3. Spectral karyotyping analysis on cultured amniocytes shows a small supernumerary marker chromosome 9 [sSMC(9)] (arrow).

Results

Metaphase FISH analysis on cultured lymphocytes of the neonate's peripheral blood confirmed coexistence of the sSMC(9) and the supernumerary r(9) (Fig. 4). The neonate's peripheral blood had the karyotype of 47,XY,+sSMC(9) [14]/48,XY,+sSMC(9),+r(9) [10]/47,XY,+r(9) [6]/46,XY [10]. SNP aCGH revealed a result of arr 9p22.3q21.11 (14,234,165–71,035,608) × 2–3 (Fig. 5), arr 9p24.3p22.3 (216,123–14,629,321)hmz (homozygosity), arr 9p21.3p13.2 (24,769,722–36,732,597)hmz and arr 9q21.11q34.3 (71,013,799–141,011,581)hmz (Fig. 6). Polymorphic DNA marker analysis confirmed paternal isodisomy 9 (Table 1).

Discussion

We have presented a very rare occurrence of paternal UPD 9 associated with mosaic sSMC(9). To our knowledge, only six paternal UPD 9 cases have been reported [17–23]. Kaiser-Rogers et al. [17] first reported the detection of paternal UPD 9 in a dizygotic female co-twin with intrauterine growth restriction (IUGR) and late pregnancy *in utero* fetal death. Van der Hagen et al. [18] reported prenatally diagnosed trisomy 9 mosaicism associated with paternal UPD 9 in a child with prenatal findings of IUGR, atrial-

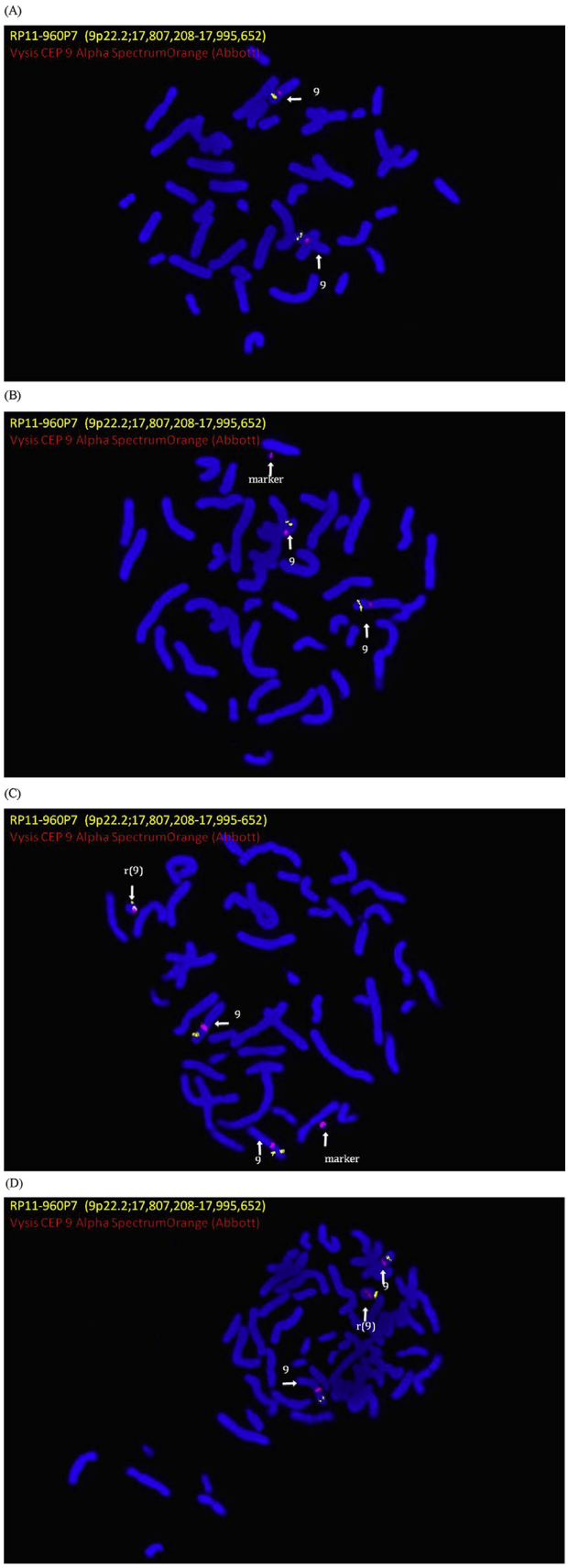

Fig. 4. Metaphase fluorescence *in situ* hybridization analysis on cultured lymphocytes using the RP11-960P7 bacterial artificial chromosome probe (9p22.2, 17,807,208–17,995,652) (yellow signal) and the Vysis CEP9 Alpha centromeric probe (red signal) shows (A) a normal cell with disomy 9 with two yellow signals and two red signals in each chromosome 9; (B) an abnormal cell with two chromosomes 9 and a marker chromosome with only one red signal in the marker chromosome; (C) an abnormal cell with two chromosomes 9, a marker chromosome and a supernumerary ring chromosome 9 [r(9)] with one yellow signal and one red signal in the supernumerary r(9); and (D) an abnormal cell with two chromosomes 9 and a supernumerary r(9).

ventricular septal defects and mosaic trisomy 9 in amniotic fluid at prenatal diagnosis, and postnatal findings of paternal heterodisomy 9 in the lymphocytes and skin fibroblasts, growth retardation, development delay and muscular hypotonia. Yang et al. [19] reported a 20-year-old female with paternal UPD 9 and juvenile amyotrophic lateral sclerosis type 16 with the affected gene of *SIGMR1* being located at 9p13.3. Carvalho et al. [20] reported paternal UPD 9 in a postnatal case with duplication and triplication in 9q12-q21.11. Ma et al. [21] reported a 22-month-old girl with severe developmental delay, congenital cerebral dysplasia, congenital heart disease, trisomy 9 mosaicism and paternal isodisomy 9. Our case adds to the list of paternal UPD 9 with no apparent phenotypic abnormalities.

The present case had molecular cytogenetic discrepancy between prenatal cultured amniocytes and postnatal peripheral blood lymphocytes. In the cultured amniocytes, aCGH detected mosaic duplication of 9p13.1q21.11, whereas in the peripheral blood, aCGH detected mosaic duplication of 9p22.3q21.11. This discrepancy is in accordance with the conventional cytogenetic discrepancy between cultured amniocytes and cultured blood lymphocytes. In the cultured amniocytes, the supernumerary r(9) appeared in 5/36 (13.9%) of the colonies, whereas in the cultured blood lymphocytes, the supernumerary r(9) appeared in 16/40 (40%) of the cells.

The present case had mosaic duplication of 9p22.3-q21.1 in the peripheral blood. Various studies have shown that duplication of 9p11.2-p13.1, 9q13-q21.12 and 9p11.2-q13.1 are euchromatic variants with no phenotypic abnormalities [10,24–31]. The 9p duplication syndrome is characterized by psychomotor and growth retardations, microcephaly, facial dysmorphism of down-slanting palpebral fissures, hypertelorism, a bulbous nose, down-turned corners of the mouth, low-set ears, short fingers and toes, hypoplastic phalanges of the fifth fingers, hypoplastic nails, genital hypoplasia, developmental delay and variable degrees of intellectual disability [32–37]. Zou et al. [36] suggested that in cases with 9p duplication syndrome, the critical region for mental retardation lies within 2.6 Mb of the 9p22.3-p23, and the critical region for speech/language delay lies within 4.9 Mb of the 9p21.2-p21.3. Haddad et al. [38] and Fujimoto et al. [33] suggested that the minimal critical region for the 9p duplication syndrome is located at 9p22-p24. The region of 9p23-p24.3 carries potential autism spectrum disorders (ASD) locus [39]. Abu-Amero et al. [39] reported a 17-year-old girl with ASD, severe mental retardation, epilepsy, partial 9p duplication syndrome and a *de novo* SMC derived from 9p13.1-p24.3. Chen et al. [40] reported a 13-year-old girl with microcephaly, facial dysmorphism, brachydactyly, neuropsychomotor developmental delay, speech delay and self-injurious behavior with trisomy 9p (9p13.1-p24.3). Chen et al. [12] reported mosaicism for an sSMC derived from 9p13.1-p23 and clinical features of attention deficit and hyperactivity disorder (ADHD), clinodactyly and gynecomastia in a 12-year-old boy. The present case had partial duplication of 9p13.1-p22.3. Bonaglia et al. [41] reported a 6½-year-old girl with minimal physical findings and normal intelligence quotient (IQ) with a partial duplication of 9p13.1-p22.1.

In summary, we present the detection of paternal UPD 9 in association with prenatally detected mosaicism for an sSMC(9) and a supernumerary r(9). Our case provides evidence that individuals

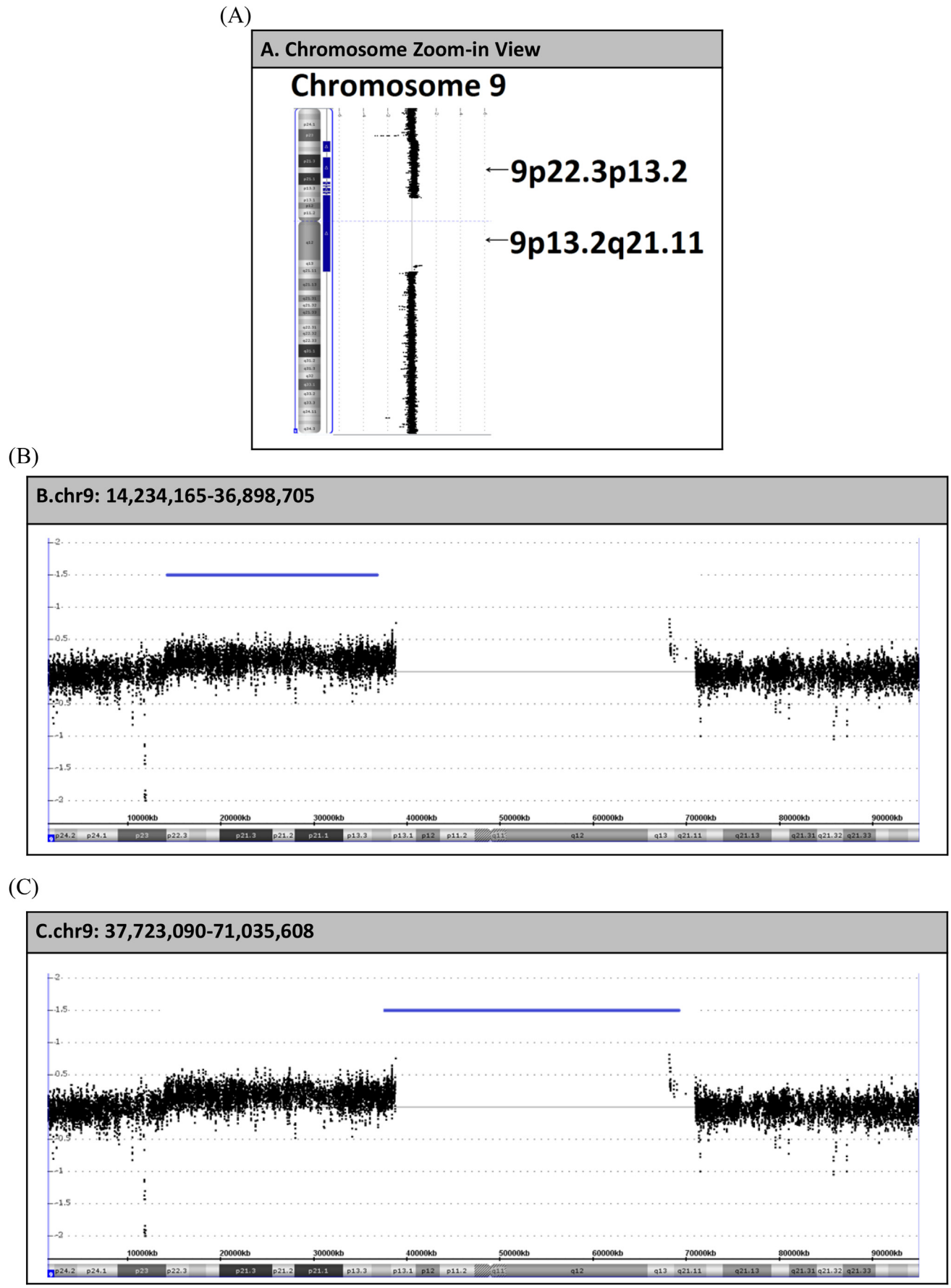

Fig. 5. Single nucleotide polymorphism array comparative genomic hybridization (SNP aCGH) analysis on the DNA extracted from the neonate's peripheral blood shows the result of arr 9p22.3q21.11 (14,234,165–71,035,608) × 2–3. (A), (B) and (C) Chromosome zoom-in views.

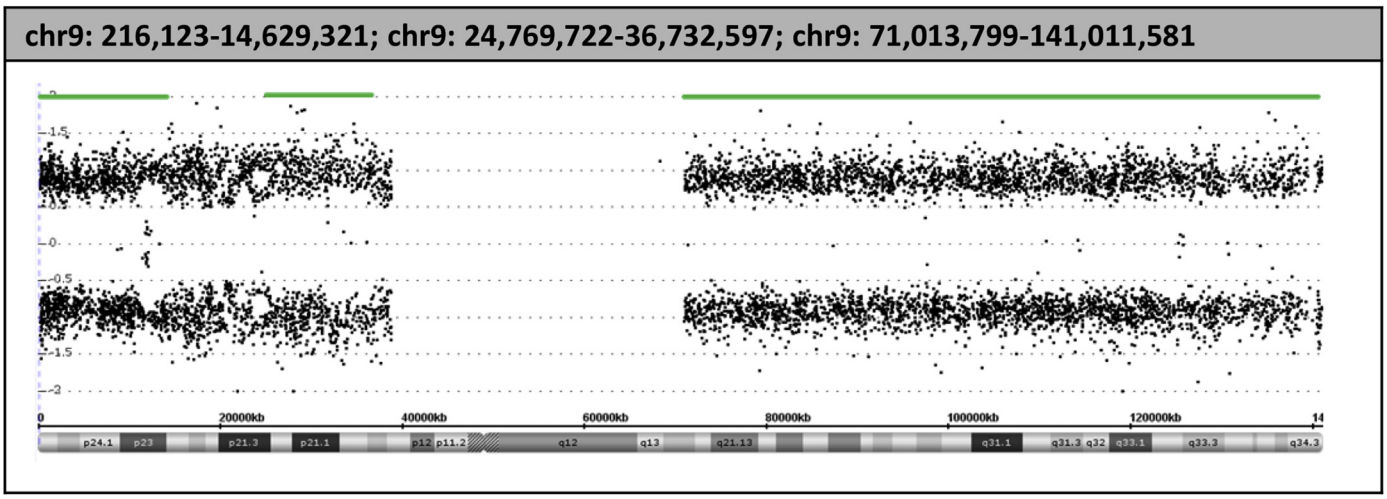

Fig. 6. SNP aCGH analysis on the DNA extracted from the peripheral blood shows the result of arr 9p24.3p22.3 (216,123–14,629,321)hmz, arr 9p21.3p13.2 (24,769,722–36,732,597) hmz and arr 9q21.11q34.3 (71,013,799–141,011,581). hmz = homozygosity.

Table 1
Molecular results using polymorphic DNA markers specific for chromosome 9.[a]

Markers	Locus	Father	Mother	Proband	Result
D9S304	9p21.1	130, 150	134, 142	130, 130	Paternal isodisomy
D9S303	9q21.32	146, 154	146, 154	146, 146	Non-informative
D9S249	9q21.33	141, 141	141, 141	141, 141	Non-informative
D9S299	9q31.2	180, 188	180, 184	188, 188	Paternal isodisomy

[a] Alleles (basepair sizes) are listed below each individual.

with mosaicism for sSMC(9) and supernumerary r(9) may be associated with paternal UPD 9.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

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References

[1] Liehr T, Claussen U, Starke H. Small supernumerary marker chromosomes (sSMC) in humans. Cytogenet Genome Res 2004;107:55–67.
[2] Callen DF, Ringenbergs ML, Fowler JCS, Freemantle CJ, Haan EA. Small marker chromosomes in man: origin from pericentric heterochromatin of chromosomes 1, 9, and 16. J Med Genet 1990;27:155–9.
[3] Callen DF, Eyre H, Yip MY, Freemantle J, Haan EA. Molecular cytogenetic and clinical studies of 42 patients with marker chromosomes. Am J Med Genet 1992;43:709–15.
[4] Miller WA, Thayer BA, McLaughlin C, et al. Chromosomal origin of *de novo* marker chromosomes identified prenatally. Am J Hum Genet 1992;51(Suppl): A1030.
[5] Blennow E, Anneren G, Bui T-H, Berggren E, Asadi E, Nordenskjold M. Characterization of supernumerary ring marker chromosomes by fluorescence *in situ* hybridization (FISH). Am J Hum Genet 1993;53:433–42.
[6] Blennow E, Nielsen KB, Telenius H, Carter NP, Kristoffersson U, Holmberg E, et al. Fifty probands with extra structurally abnormal chromosomes characterized by fluorescence *in situ* hybridization. Am J Med Genet 1995;55:85–94.
[7] James RS, Temple IK, Dennis NR, Crolla JA. A search for uniparental disomy in carriers of supernumerary marker chromosomes. Eur J Hum Genet 1995;3: 21–6.
[8] Müller-Navia J, Nebel A, Schleiermacher E. Complete and precise characterization of marker chromosomes by application of microdissection in prenatal diagnosis. Hum Genet 1995;96:661–7.
[9] Anderlid BM, Sahlen S, Schoumans J, Holmberg E, Ahsgren I, Mortier G, et al. Detailed characterization of 12 supernumerary ring chromosomes using micro-FISH and search for uniparental disomy. Am J Med Genet 2001;99: 223–33.
[10] Starke H, Seidel J, Henn W, Reichardt S, Volleth M, Stumm M, et al. Homologous sequences at human chromosome 9 bands p12 and q13-21.1 are involved in different patterns of pericentric rearrangements. Eur J Hum Genet 2002;10:790–800.
[11] Guanciali-Franchi P, Calabrese G, Morizio E, Fantasia D, Colosimo A, Rinaldi MM, et al. Identification of 14 rare marker chromosomes and derivatives by spectral karyotyping in prenatal and postnatal diagnosis. Am J Med Genet 2004;127A:144–8.
[12] Chen C-P, Lin S-P, Su J-W, Town D-D, Wang W. A *de novo* supernumerary marker chromosome derived from chromosome 9p (9p13.1→p23) associated with attention deficit and hyperactivity disorder. Genet Couns 2012;23: 329–33.
[13] Hamid AB, Kreskowski K, Weise A, Kosayakova N, Mrasek K, Voigt M, et al. How to narrow down chromosomal breakpoints in small and large derivative chromosomes – a new probe set. J Appl Genet 2012;53:259–69.
[14] Liehr T, Klein E, Mrasek K, Kosyakova N, Guilherme RS, Aust N, et al. Clinical impact of somatic mosaicism in cases with small supernumerary marker chromosomes. Cytogenet Genome Res 2013;139:158–63.
[15] Lo KK, Karampetsou E, Boustred C, McKay F, Mason S, Hill M, et al. Limited clinical utility of non-invasive prenatal testing for subchromosomal abnormalities. Am J Hum Genet 2016;98:34–44.
[16] Liehr, T. Small supernumerary marker chromosomes: chromosome 9. Available at: http://ssmc-tl.com/chromosome-9.html. [Accessed 20 February 2017].
[17] Kaiser-Rogers KA, Robinson WP, Knops JF, Vargo D, Livasy CA, Bailit J, et al. A rare case of mosaicism for paternal UPD 9 in a dizygotic twin pregnancy. Am J Hum Genet 2002;71(Suppl 4):305.
[18] Van der Hagen CB, Eiklid K, Ørstavik KH, Braaten O, Prescott T, Kroken M, et al. Prenatally diagnosed trisomy 9 mosaicism and paternal uniparental disomy 9 in the child. Eur J Hum Genet 2003;11(Suppl):P409.
[19] Yang Y, Muzny DM, Xia F, Niu Z, Person R, Ding Y, et al. Molecular findings among patients referred for clinical whole-exome sequencing. JAMA 2014;312:1870–9.
[20] Carvalho CMB, Pfundt R, King DA, Lindsay SJ, Zuccherato LW, Macville MVE, et al. Absence of heterozygosity due to template switching during replicative rearrangements. Am J Hum Genet 2015;96:555–64.
[21] Ma J, Cram DS, Zhang J, Shang L, Yang H, Pan H. Birth of a child with trisomy 9 mosaicism syndrome associated with paternal isodisomy 9: case of a positive noninvasive prenatal test result unconfirmed by invasive prenatal diagnosis. Mol Cytogenet 2015;8:44.
[22] Joshi RS, Garg P, Zaitlen N, Lappalainen T, Watson CT, Azam N, et al. DNA methylation profiling of uniparental disomy subjects provides a map of parental epigenetic bias in the human genome. Am J Hum Genet 2016;99: 555–66.
[23] Liehr, T. Cases with uniparental disomy. Available at: http://ssmc-tl.com/upd.html. [Accessed 01 March 2017].
[24] Madan K. An extra band in human 9qh+ chromosomes. Hum Genet 1978;43: 259–64.
[25] Jalal SM, Kukolich MK, Garcia M, Day DW. Euchromatic 9q+ heteromorphism in a family. Am J Med Genet 1990;37:155–6.

[26] Knight LA, Soon MG, Tan M. Extra G positive band on the long arm of chromosome 9. J Med Genet 1993;30:613.
[27] Reddy KS. Variants of chromosome 9 with additional euchromatic bands: two case reports. Am J Med Genet 1996;64:536–8.
[28] Park JP, Wojiski SA, Spellman RA, Rhodes CH, Mohandas TK. Human chromosome 9 pericentric homologies: implications for chromosome 9 heteromorphisms. Cytogenet Cell Genet 1998;82:192–4.
[29] Di Giacomo MC, Cesarano C, Bukvic N, Manisali E, Guanti G, Susca F. Duplication of 9 p11.2–p13.1: a benign cytogenetic variant. Prenat Diagn 2004;24:619–22.
[30] Lecce R, Murdolo M, Gelli G, Steindl K, Coppola L, Romano A, et al. The euchromatic 9p+ polymorphism is a locus-specific amplification caused by repeated copies of a small DNA segment mapping within 9p12. Hum Genet 2006;118:760–6.
[31] Willatt LR, Barber JCK, Clarkson A, Simonic I, Raymond FL, Docherty Z, et al. Novel deletion variants of 9q13-q21.12 and classical euchromatic variants of 9q12/qh involve deletion, duplication and triplication of large tracts of segmentally duplicated pericentromeric euchromatin. Eur J Hum Genet 2007;15:45–52.
[32] Wilson GN, Raj A, Baker DD. The phenotypic and cytogenetic spectrum of partial trisomy 9. Am J Med Genet 1985;20:277–82.
[33] Fujimoto A, Lin MS, Schwartz S. Direct duplication of 9p22→p24 in a child with duplication 9p syndrome. Am J Med Genet 1998;77:268–71.
[34] Tsezou A, Kitsiou S, Galla A, Petersen MB, Karadima G, Syrrou M, et al. Molecular cytogenetic characterization and origin of two de novo duplication 9p cases. Am J Med Genet 2000;91:102–6.
[35] Temtamy S, Kamel AK, Ismail S, Helmy NA, Aglan MS, El Gammal M, et al. Phenotypic and cytogenetic spectrum of 9p trisomy. Genet Couns 2007;18:29–48.
[36] Zou YS, Huang XL, Ito M, Newton S, Milunsky JM. Further delineation of the critical region for the 9p-duplication syndrome. Am J Med Genet 2009;149A:272–6.
[37] Guilherme RS, Meloni VA, Perez ABA, Pilla AL, de Ramos MAP, Dantas AG, et al. Duplication 9p and their implication to phenotype. BMC Med Genet 2014;15:142.
[38] Haddad BR, Lin AE, Wyandt H, Milunsky A. Molecular cytogenetic characterisation of the first familial case of partial 9p duplication (p22p24). J Med Genet 1996;33:1045–7.
[39] Abu-Amero KK, Hellani AM, Salih MA, Seidahmed MZ, Elmalik TS, Zidan G, et al. A *de novo* marker chromosome derived from 9p in a patient with 9p partial duplication syndrome and autism features: genotype-phenotype correlation. BMC Med Genet 2010;11:135.
[40] Chen C-P, Lin S-P, Su Y-N, Chern S-R, Tsai F-J, Chen W-L, et al. Self-injurious behavior associated with trisomy 9p (9p13.1→p24.3). Genet Couns 2011;22:327–31.
[41] Bonaglia MC, Giorda R, Carrozzo R, Roncoroni ME, Grasso R, Borgatti R, et al. 20-Mb duplication of chromosome 9p in a girl with minimal physical findings and normal IQ: narrowing of the 9p duplication critical region to 6 Mb. Am J Med Genet 2002;112:154–9.